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(54) **SENSOR**

SENSOR

DETECTEUR

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(56) References cited:
**WO-A-91/08474 US-A- 4 762 594
US-A- 4 963 245 US-A- 5 120 421**

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Description

[0001] This invention relates to sensors for analytes present in solution.

[0002] Conductimetric sensors based on redox state dependent conducting organic polymers such as polypyrrole, 5 polyaniline and polyindole are well known. Such sensors commonly comprise a layer of the conducting organic polymer bridging two closely spaced electrodes, and commonly rely upon the detection of changes in the resistance or ac impedance of the polymer. This change in resistance is caused by the interaction between the analyte - which may be present in solution or in the gas phase - and the polymer. An important area of application lies in the incorporation of enzymes into the organic polymer to produce 'biosensors' capable of quantitative sensing of biologically significant 10 analytes. Exposure of the enzyme to the analyte induces catalytic activity which in turn induces conductivity changes within the enzyme trapping polymer matrix. Although the precise nature of the conductivity changes are unclear - and may well differ with different combinations of polymer and enzyme - local H⁺ concentration effects and, in the case of oxidases, ketones and aldehydes, H₂O₂ generation have been implicated. Broadly speaking, the conductivity of the 15 polymer is perturbed by the redox behaviour of the enzyme on exposure to the analyte. Since enzyme-substrate interactions are typically very specific, the associated biosensor represents a highly selective method of analyte detection.

[0003] However, it is usually only possible to bridge the electrodes by electrochemical growth of organic polymer if the electrodes are rather closely spaced (typically < 20μm). This upper limit imposes restrictions on the magnitude of the measured signal and also requires precise electrode geometries which in turn requires technically demanding and expensive fabrication procedures such as screen printing or silicon chip fabrication techniques.

20 [0004] An appealing alternative which circumvents the problem of precise electrode alignment would involve measuring the impedance or admittance of a polymer bridging an analyte solution and a planar working electrode; in such an arrangement measurements would be made in the {working electrode → polymer → analyte solution → counter electrode} plane. Detection of the analyte would be accomplished by detecting changes in the impedance or admittance of the polymer induced by interaction between the polymer and the analyte. However, the linear mass transport regime 25 operating in this arrangement would inevitably result in a detection signal of insufficient magnitude for useful measurement.

[0005] The present invention is based upon the concept of incorporating within the above described arrangement a working electrode assembly which comprises an array of conducting organic polymer coated micro-electrodes. Such an electrode results in radial transport of the analyte, rendering feasible the use of the arrangement as a sensor.

30 [0006] The present invention further provides examples of such arrays of micro-electrodes and methods of fabricating same.

[0007] International Publication WO 91/08474 describes a method for fabricating a microelectrode by photoablation. The microelectrode was used in an amperometric assay method for the detection of heavy metals.

-35 [0008] According to one aspect of the invention there is provided a sensor for an analyte comprising a working electrode assembly itself comprising a micro-electrode array in which each micro-electrode is coated with a layer of redox state dependent conducting organic polymer; a counter electrode; a conductive medium containment into which the electrodes are disposed and a material for analysis may be introduced ; means for applying an alternating electric polarising potential across the electrodes; and means for detecting a variation in a conductimetric property across the {working electrode → counter electrode} plane in the presence of the analyte.

40 [0009] The conductive medium containment may comprise a conducting solution.

[0010] The conductimetric property may be the impedance across the {working electrode → counter electrode} plane.

[0011] The variation in the conductimetric property may be detected as a function of the applied frequency.

[0012] Alternatively, said variation may be detected at a single applied frequency.

45 [0013] The working electrode may be fabricated by coating a planar electrode with an insulating polymer, sonically ablating the insulating polymer coating to produce a plurality of micro-pores, and depositing conducting organic polymer into said micro-pores. The conducting organic polymer may be deposited electrochemically or by other means such as chemical vapour deposition, photopolymerisation techniques or other thin or thick film surface coating technologies.

[0014] An enzyme may be entrapped within the semiconducting organic polymer matrices. The semiconducting organic polymer may be polyaniline, polypyrrole, polyindole or equivalents thereof. The enzyme may be a redox enzyme 50 such as glucose oxidase, alcohol oxidase and alcohol dehydrogenase. Further, the planar electrode may be a noble metal or a conducting material such as carbon and the insulating polymer may be polydiaminobenzenedihydrochloride, Teflon, PVC or polyethylvinylbenzene.

[0015] According to a second aspect of the invention there is provided a method of fabricating a working electrode assembly comprising coating a planar electrode with an insulating polymer, sonically ablating the insulating polymer coating to produce a plurality of micro-pores, and depositing redox state dependent conducting organic polymer into said microphones.

[0016] The organic polymer may be deposited electrochemically, by chemical vapour deposition, photopolymerisation techniques or other thin or thick film surface coating technologies.

[0017] An enzyme may be entrapped within the organic polymer matrices.

[0018] Sensors and methods of fabricating working electrode assemblies in accordance with the invention will now be described with reference to the accompanying drawings in which:-

5 Figure 1 is a schematic illustration of the electrode arrangement;

Figure 2 is a schematic illustration of the control arrangement;

10 Figure 3 shows a possible equivalent circuit; and

Figure 4 is a graph of typical log (impedance) vs log (frequency) profiles at different analyte concentrations.

[0019] Figures 1 and 2 depict a sensor for detecting an analyte according to the present invention, which comprises a working electrode assembly 10, a counter electrode 12 and a conducting solution 14, wherein the working electrode assembly 10 comprises a micro-electrode array in which each micro-electrode is coated with a layer of redox state dependent conducting organic polymer. The sensor further comprises a frequency response analyser 16 which i) applies an alternating electric polarising potential across the electrode arrangement 18 and ii) detects any variation in a chosen conductimetric property across the {working electrode substrate → polymer → solution → counter electrode} plane when the analyte is present in the solution. The variation may alternatively be detected by a phase sensitive detector. Control of the frequency response analyser and collection of data may be effected *inter alia* by a computer 20 via a suitable interface.

[0020] The conductimetric sensor of the present invention utilises redox state dependent conducting organic polymer as the active, analyte sensing medium, but represents a very different detection arrangement to prior art conductimetric devices which employ polymers of this type. In particular, prior art devices typically comprise closely spaced electrodes bridged by conducting organic polymer, close in this context being about 15-20 µm or less. This approximate upper limit is due to difficulty of effectively bridging greater electrode separations with electrochemically grown polymer - by far the most commonly employed method of depositing the conducting polymer. Physically, the reason for this difficulty is that the separation of the electrodes exceeds the length of the polymeric chains, resulting in very high resistances and poor mechanical qualities. Consequently, precise electrode geometries are required, necessitating the use of technically demanding and expensive fabrication procedures such as screen printing or silicon chip fabrication techniques.

[0021] The arrangement of the present invention does not utilise conducting polymer as an electrode bridge, and thus does not require precise alignment of the electrodes. Furthermore, it is noted that use of a conventional planar working electrode coated with semiconducting organic polymer would result in linear mass transport of the analyte, which in turn would result in an unfeasibly small detection signal. With the introduction of micro-electrodes the mass transport of analyte becomes radial in nature, permitting analyte detection with excellent sensitivity.

[0022] Detection of analyte may be achieved by detecting changes in the complex plane impedance between the counter electrode and the array of polymer coated micro-electrodes which comprise the working electrode. However, detection of other conductimetric properties, such as conductance or susceptance, are also within the scope of the invention. In any event, it is necessary to separate the desired measurement of analyte induced changes in, for example, polymer resistance from the other conductimetric components of the system. Figure 3 shows a Randles equivalent circuit, which indicates that the conductimetric behaviour between the counter electrode and the working electrode may be decomposed into:

45 i) the uncompensated solution resistance R_u ,

ii) the capacitance due to the electrical double layer C_{dl} ,

50 iii) the reaction resistance, which may in turn comprise a Warburg impedance W and a charge transfer resistance R_{ct} ,

iv) the capacitance of the polymer layer C_{pf} .

The Warburg impedance W is negligible at high excitation frequencies (typically > 100Hz) and thus R_{ct} and C_{pf} , which are modulated by the analyte-polymer interaction, may be extracted.

[0023] A schematic illustration of the way in which impedance may vary as a function of applied frequency and analyte concentration is shown in Figure 4. The response curves a, b, c, d are in order of increasing analyte concentration. The presence of analyte may be detected by monitoring changes in the response function over a range of applied

frequencies. Alternatively, it may prove expedient to detect changes in impedance at a fixed frequency, this frequency advantageously being one at which particularly large impedance changes are induced by the analyte-polymer combination in question, such as the frequency marked A in Figure 4. The computer 20 may be used to store the response or response function obtained with no analyte present in the solution 14 as a reference signal, and to compare this reference signal with the response or response function obtained in the presence of an analyte.

[0024] Fourier transform analysis techniques are also applicable. However, it should be noted that a dc measurement of bulk sensor resistance would not suffice, since the dominant contribution to this resistance would be due to charging of the capacitances within the circuit, giving rise to a drifting value.

[0025] The invention also provides examples of arrays of semiconducting organic polymer coated micro-electrodes and methods of fabricating same. Generally speaking, working electrodes of the present invention may be fabricated by coating a planar electrode with an insulating polymer, sonically ablating the insulating polymer coating to produce a plurality of micro-pores, and depositing conducting organic polymer into the micro-pores. It has been shown (NA Madigan, CRS Hagan and LA Coury, J. Electrochem. Soc., 141 (1994) 1014) that micro-electrode arrays may be produced by sonicating insulating polymer coated electrodes, but the coating of the micro-electrodes with conducting organic polymer and the use of such a micro-electrode array as a sensor has no antecedent. Examples of insulating polymer films include polydiaminobenzenedihydrochloride, PVC, Teflon, polyethylvinylbenzene and the like. Examples of substrate electrode materials include gold, platinum, ruthenium, gold/platinum alloys and glassy carbon. Commonly employed solvents such as water, decane and dioxane may be used for sonication.

[0026] The conducting organic polymer may be deposited electrochemically, the precise details of the polymerisation process not varying substantially from well known literature methods. (see for example JC Cooper and EAH Hall, Biosensors & Bioelectronics, 7 (1992) 473) Examples of electropolymerisable conducting organic polymers include polyaniline, polypyrrole, polyindole and poly-N-methylpyrrole, although it is to be understood that this list is a non-limiting one: many examples of suitable conducting organic polymers may be found in the literature. Other deposition means, such as chemical vapour deposition, photopolymerisation techniques or other thin or thick film surface coating technologies are within the scope of the invention.

[0027] An important aspect of the present invention lies in the incorporation of enzymes into the conducting organic polymer to produce biosensors capable of sensing of, for example, biologically significant analytes. The incorporation of enzymes into the polymer may be accomplished by any of the many well characterised methods documented in the literature. For example, the polymer may be electrochemically polymerised from a solution containing both monomer and enzyme.

[0028] A specific example of a sensor of the present invention is a device sensitive to the presence of glucose. The working electrode was produced by sonication in water of a gold planar electrode coated with polydiaminobenzenedihydrochloride, followed by electropolymerisation of aniline in the presence of glucose oxidase. Sonication is typically at 15KHz for one minute. Glucose oxidase catalyses the oxidation of glucose, i.e:

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[0029] The choice of polyaniline as the conducting organic polymer is influenced by its stability in the presence of hydrogen peroxide. However, it should be noted that the use of other conducting organic polymers and other enzymes is within the scope of the invention. Measurements of impedance profile as a function of applied frequency at varying concentrations of the glucose analyte (1-20mM glucose in a pH 7 phosphate buffer) have been made using a glucose sensor of this type. The results indicate that change in an impedance value measured at a chosen frequency (compared to the impedance obtained at the same frequency with no glucose present) is proportional to the concentration of glucose in the solution in the low (<5mM) concentration regime. As an example, measurements made at a frequency of 11.3 Hz show that the impedance of the polymer film is ca. 400 Ω in the absence of glucose, but in the presence of a 5 mM glucose solution this impedance drops to ca. 200 Ω. This represents an extremely large signal, well above the detection limit of the apparatus employed, which is capable of detecting changes in resistance of the order of 0.1Ω or less.

[0030] Similar results have been obtained with a sensor incorporating alcohol oxidase as the enzyme; ethanol is the analyte in this instance. This demonstrates that the present invention may be applied to different analyte and enzyme combinations. For ethanol concentrations of 15 mM, a drop in impedance from ca. 400 Ω to ca. 200 Ω has been observed.

[0031] Many further variations within the scope and spirit of the invention are possible. For instance, it may be desirable to combine several working electrodes within one device, wherein each working electrode comprises a different polymer and / or enzyme. In one example each working electrode would have a different enzyme incorporated into the

polymer matrix, and each enzyme would be sensitive to a different analyte. The resulting device would be sensitive to the presence of a number of specific analytes. Alternatively, it is well known that semiconducting organic polymer based conductimetric sensors which do not incorporate analyte reception agents such as enzymes are usually sensitive to a range of analytes. Therefore, with prior art devices which rely upon the measurement of changes in dc resistance, precise identification of an analyte with a single sensor incorporating a single polymer can present a problem . The present invention is advantageous in this respect, since the response of the sensor as a function of frequency can provide an extra detection dimensionality compared to a single, bulk, dc resistance measurement, and may be considered a 'fingerprint' of the analyte detected. However, it may be desirable to produce a device having a plurality of working electrodes which each involve a different semiconducting organic polymer ; in such a device the pattern of response across the plurality of working electrodes may be indicative of a particular analyte. Indeed, it may prove possible to deduce the individual components of a mixture from the response patterns of such a device.

Claims

1. A sensor for an analyte comprising a working electrode assembly (10) itself comprising a micro-electrode array in which each micro-electrode is coated with a layer of redox state dependent conducting organic polymer; a counter electrode (12); a conductive medium (14) containment into which the electrodes are disposed and a material for analysis may be introduced ; means for applying an alternating electric polarising potential (16) across the electrodes; and means for detecting (16) a variation in a conductimetric property across the {working electrode → counter electrode} plane in the presence of the analyte.
2. A sensor according to claim 1 in which the conductive medium containment comprises a conducting solution.
3. A sensor according to claim 1 or claim 2 in which the conductimetric property detected is the impedance across the {working electrode → counter electrode} plane.
4. A sensor according to any of the previous claims in which the variation in the conductimetric property is detected as a function of the applied frequency.
5. A sensor according to any of the previous claims in which the variation in the conductimetric property is detected at a single applied frequency.
6. A sensor according to any of the previous claims in which the working electrode is fabricated by coating a planar electrode with an insulating polymer, sonically ablating the insulating polymer coating to produce a plurality of micro-pores, and depositing redox state dependent organic polymer into said micro-pores.
7. A sensor according to claim 6 in which the conducting organic polymer is deposited electrochemically, by chemical vapour deposition or by photopolymerisation.
8. A sensor according to any of the previous claims in which an enzyme is entrapped within the conducting organic polymer matrices.
9. A sensor according to claim 8 in which the enzyme is glucose oxidase, alcohol oxidase or alcohol dehydrogenase.
10. A sensor according to any of claims 6 to 9 in which the insulating polymer is polydiaminobenzenedihydrochloride, Teflon, PVC or polyethylvinylbenzene.
11. A method of fabricating a working electrode assembly comprising coating a planar electrode with an insulating polymer, sonically ablating the insulating polymer coating to produce a plurality of micro-pores, and depositing redox state dependent conducting organic polymer into said micro-pores.
12. A method according to claim 11 in which the organic polymer is deposited electrochemically, by chemical vapour deposition or by photopolymerisation.
13. A method according to claim 11 or claim 12 in which an enzyme is entrapped within the organic polymer matrices.

Patentansprüche

1. Sensor für einen Analyten mit einer Arbeitselektrodenanordnung (10), die selbst eine Mikroelektrodenanordnung aufweist, in der jede Mikroelektrode mit einer Schicht aus vom Redox-Zustand abhängigem, leitendem organischem Polymer bedeckt ist; einer Gegenelektrode (12); einem eingeschlossenen leitenden Medium (14), in welchem die Elektroden angeordnet sind und in welches ein Material für die Analyse eingeführt werden kann; Mitteln zum Anlegen einer polarisierenden elektrischen Wechselspannung (16) an die Elektroden; und Mitteln zum Erfassen (16) einer Veränderung in einer konduktometrischen Eigenschaft über die {Arbeitselektroden → Gegenelektroden}-Ebene in Anwesenheit des Analyten.
2. Sensor nach Anspruch 1, in welchem das eingeschlossene leitende Medium eine leitende Lösung aufweist.
3. Sensor nach Anspruch 1 oder Anspruch 2, in welchem die erfaßte konduktometrische Eigenschaft die Impedanz über die {Arbeitselektroden → Gegenelektroden}-Ebene ist.
4. Sensor nach einem der vorhergehenden Ansprüche, in welchem die Veränderung der konduktometrischen Eigenschaft als eine Funktion der angelegten Frequenz erfaßt wird.
5. Sensor nach einem der vorhergehenden Ansprüche, in welchem die Veränderung der konduktometrischen Eigenschaft bei einer einzigen angelegten Frequenz erfaßt wird.
6. Sensor nach einem der vorhergehenden Ansprüche, in welchem die Arbeitselektrode hergestellt ist durch Beschichten einer planaren Elektrode mit einem isolierenden Polymer, akustischen Ablatieren der isolierenden Polymerbeschichtung, um eine Vielzahl von Mikroporen zu erzeugen, und Ablagern von vom Redox-Zustand abhängigem organischem Polymer in den Mikroporen.
7. Sensor nach Anspruch 6, in welchem das leitende organische Polymer elektrochemisch, durch chemische Gasphasenabscheidung oder durch Photopolymerisation abgelagert ist.
8. Sensor nach einem der vorhergehenden Ansprüche, in welchem ein Enzym in die leitenden organischen Polymermatrizen eingeschlossen ist.
9. Sensor nach Anspruch 8, in welchem das Enzym Glukoseoxidase, Alkoholoxidase oder Alkoholdehydase ist.
10. Sensor nach einem der Ansprüche 6 bis 9, in welchem das isolierende Polymer Polydiaminobenzoldihydrochlorid, Teflon, PVC oder Polyethylvinylbenzol ist.
11. Verfahren zum Herstellen einer Arbeitselektrodenanordnung, das das Beschichten einer planaren Elektrode mit einem isolierenden Polymer, das akustische Ablatieren der isolierenden Polymerbeschichtung, um eine Vielzahl von Mikroporen zu erzeugen, und das Ablagern von vom Redox-Zustand abhängigem, leitendem organischem Polymer in den Mikroporen umfaßt.
12. Verfahren nach Anspruch 11, bei welchem das organische Polymer elektrochemisch, durch chemische Gasphasenabscheidung oder durch Photopolymerisation abgeschieden ist.
13. Verfahren nach Anspruch 11 oder Anspruch 12, bei welchem ein Enzym in den organischen Polymermatrizen eingeschlossen ist.

Revendications

1. DéTECTEUR pour analyte comprenant un assemblage d'électrodes de travail (10) comprenant lui-même une rangée de micro-électrodes dans laquelle chaque micro-électrode est revêtue d'une couche de polymère organique conducteur dépendant de l'état redox; une électrode de comptage (12), un milieu conducteur (14) contenu dans lequel les électrodes sont disposées et un matériau pour analyse puisse être introduit ; des moyens pour appliquer un potentiel polarisant électrique alternatif (16) à travers les électrodes ; et des moyens pour détecter (16) une variation dans une propriété conductimétrique à travers le plan (électrode de travail — électrode de comptage) en présence de l'analyte.

2. Détecteur selon la revendication 1, dans lequel le milieu conducteur contenu comprend une solution conductrice.
3. Détecteur selon la revendication 1 ou la revendication 2, dans lequel la propriété conductimétrique détectée est l'impédance à travers le plan {électrode de travail — électrode de comptage}.
- 5 4. Détecteur selon l'une des revendications précédentes, dans lequel la variation de la propriété conductimétrique est détectée comme une fonction de la fréquence appliquée.
- 10 5. Détecteur selon l'une quelconque des revendications précédentes, dans lequel la variation de la propriété conductimétrique est détectée à une fréquence appliquée unique.
- 15 6. Détecteur selon l'une quelconque des revendications précédentes, dans lequel l'électrode de travail est fabriquée par revêtement d'une électrode planaire par un polymère d'isolation, l'ablation par ultrasons du revêtement polymère d'isolation pour produire une pluralité de micro-pores, et le dépôt du polymère organique dépendant de l'état redox dans lesdits micro-pores.
7. Détecteur selon la revendication 6, dans lequel le polymère organique conducteur est déposé électrochimiquement, par dépôt de vapeur chimique ou par photopolymérisation.
- 20 8. Détecteur selon l'une quelconque des revendications précédentes, dans lequel une enzyme est piégée dans des matrices de polymère organique conducteur.
9. Détecteur selon la revendication 8, dans lequel l'enzyme est une glucoseoxydase, une alcooloxydase ou une alcooldéshydrogénase.
- 25 10. Détecteur selon l'une quelconque des revendications 6 à 9, dans lequel le polymère d'isolation est le poly(dichlorhydrate de diaminobenzène), le téflon, le PVC ou le polyéthylvinylbenzène.
11. Procédé de fabrication d'un assemblage d'électrodes de travail comprenant le revêtement d'une électrode planaire par un polymère d'isolation, l'ablation par ultrasons du revêtement de polymère d'isolation pour produire une pluralité de micro-pores, et le dépôt de polymère organique conducteur dépendant de l'état redox dans lesdits micro-pores.
- 30 12. Procédé selon la revendication 11, dans lequel le polymère organique est déposé électrochimiquement par dépôt de vapeur chimique ou par photopolymérisation.
13. Procédé selon la revendication 11 ou la revendication 12, dans lequel une enzyme est piégée dans les matrices de polymère organique.

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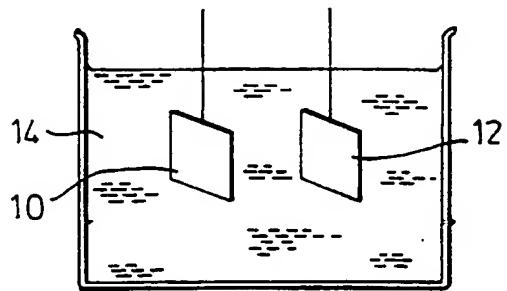


FIG.1

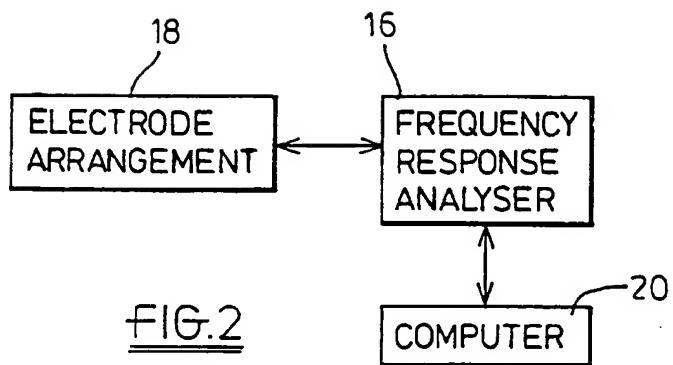


FIG.2

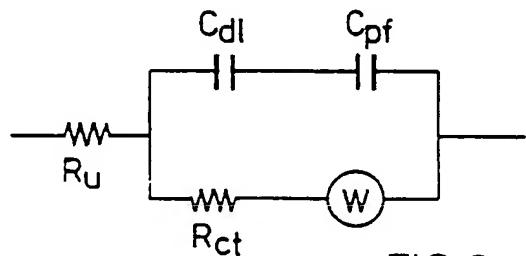


FIG.3

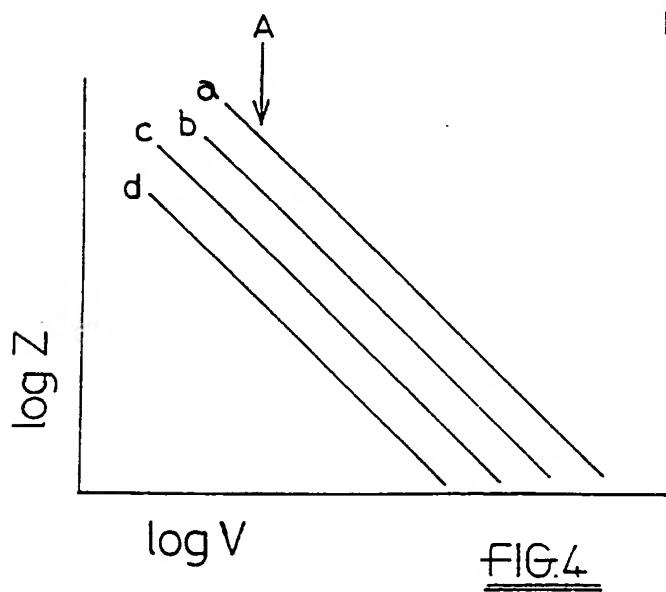


FIG.4